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PICRO-NIGROSIN, A COMBINATION FIXATIVE AND STAIN FOR ALGAE¹

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Pfitzer (1) used a saturated aqueous solution of picric acid, to which he added a varying amount of an aqueous solution of nigrosin, to kill and stain minute forms, such as diatoms and small algae, under the cover glass. Freeborn (2), Pianese (3), and Johnston (4), and other zoological investigators have employed picro-nigrosin alone, or in combination with other stains, with excellent success, but there is little reference to its use by botanists. The writers, by independent study, have found it a valuable stain for filamentous algae and for younger tissues of forms with denser structure, and believe that the results of their experiments, which confirm Pfitzer's results in practically every detail, are of sufficient interest to warrant this brief note.

The combination fixing and staining solution, which may be used several times without apparent deterioration, was prepared by saturating a saturated aqueous solution of picric acid with Gruebler's water-soluble nigrosin. Material was placed in this solution for from three to twenty-four hours, rinsed in water to remove the excess stain, and run through the grade alcohols with half hour intervals, to glycerine, clove oil, Venetian turpentine or balsam. The general result, regardless of variations in the method after the material has been run through the alcohols, is a blue-black coloration of the nucleus and nucleolus, a faint blue in the pyrenoid and cytoplasm, an almost colorless chromatophore, and a colorless cell wall. The stain is unusually transparent, differentiating the nuclear structure in cells and tissues with dense cytoplasm. For immediate class use, algae were studied in 70 per cent alcohol or transferred to a mixture of alcohol and glycerine which served to clear fairly well and prevented the mount from drying up during the laboratory exercise. Some of the best results were obtained with material transferred from absolute alcohol to clove oil and examined in the latter. Excellent permanent mounts were made by following Chamberlain's Venetian turpentine method

¹ Contribution from the Marine Biological Laboratory, Woods Hole, Massachusetts.

(5), or by running through the grades of xylol into a weak solution of balsam in xylol, which was then allowed to thicken gradually to the right consistency for mounting. The stain keeps well in both media. Shrinkage and distortion, especially in young cells, is practically negligible when reasonable care is used in handling material. For class use the Cyanophyceae, Nematium, and forms without large vacuoles can be mounted directly in balsam from clove oil or xylol with little or no shrinkage. Forms with large vacuoles may show some distortion when transferred from absolute alcohol to clove oil or xylol but the amount of shrinkage may be decreased by making the change more gradually. Apparently there is no need of carefully timed treatment as there is rarely any danger of injury or overstain; for, with most material, the results vary little whether the time of fixing and staining be twelve hours or three days. A few forms, particularly some of the red algae, should rarely be left in the solution more than twelve hours as they soften very quickly; other forms, although they may seem to have been softened and spoiled are hardened and restored to normal appearance in the higher alcohols. Pfitzer suggests the use of ammonia to take out the excess stain, but it has been found sufficient in most cases to rinse in water or simply to lengthen the time in the lower alcohols.

Experiments were conducted to determine the value of the combination killing and staining solution on widely separated genera of the algae, including *Oscillatoria*, *Lyngbya*, *Volvox*, *Spirogyra*, *Zygnema*, *Oedogonium*, *Ulva*, *Enteromorpha*, *Nitella*, *Ectocarpus*, *Sphacelaria*, *Fucus*, *Nematium*, *Callithamnion*, *Ceramium* and *Polysiphonia*, and on various Diatoms, several genera of the Agaricaceae, and young root tips. The stain proved very satisfactory for the algae, with the exception of *Ceramium* and *Polysiphonia*, and the Diatoms, but further work must be done with fungi and higher plant tissues before definite conclusions can be stated.

The value of the method may be indicated by a brief statement of the general results on some of the forms studied: In *Oscillatoria* and *Lyngbya* the chromatin granules are brought out distinctly because they stain more deeply than the rest of the cell contents. In *Enteromorpha* the nucleus is clearly differentiated; the pyrenoid is stained faintly and appears to stand in a colorless area, the chromatophore, which is hardly stained at all. In *Sphacelaria* the young propagulae show practically no shrinkage, even when material is removed from

absolute alcohol to clove oil and then to balsam. The general nuclear phenomena of propagula-formation may be followed without sectioning. In unfertilized *Fucus* eggs the nucleus stands out sharply, surrounded by radiating cytoplasmic strands. In fertilized eggs, since the brown color is taken out, one can follow the enlargement of the nucleus and its elongation, the appearance of the centrosomes and the spindle figure, and the reorganization of the daughter nuclei. The actual fusion of the egg and sperm nucleus is difficult to observe. In the two weeks old embryo, the structure of the multicellular body resulting from the division of the fertilized egg shows well; in the rapidly growing rhizoidal tips, the spindle figures are nicely differentiated. In *Nitella* the nucleus and nucleolus in the ripe oosphere are clearly visible through the spiral corticating cells. The latter, as well as the young cells and growing tips, show practically no shrinkage. In all cases diatoms are cleared so that the nucleus and general structure may be studied with ease.

Further work is being carried on with fungi and with other material which indicates that the combination killing, fixing and staining solution may have a much wider range of usefulness. The experiments so far conducted apparently establish the following points of special advantage:—

There is an unusually wide range in the time of treatment,—three hours to three days; but, even in the long period, there is little danger of overstaining or overfixing.

Since excess stain and acid are removed in most cases by rinsing in water or in the process of running through the alcohols no special washing is required.

The stain is quite insoluble in strong alcohol; therefore material may be kept for a long time in the higher alcohols.

It is an exceptionally transparent stain which differentiates the nuclear structure in cells with dense cytoplasm.

The fixation is usually good and if sufficient care is taken in subsequent treatment excellent permanent mounts can be made.

The stain keeps well in Venetian turpentine or balsam.

These facts suggest that the method, which is unusually simple, may be valuable in preparing material for class work and in preliminary cytological work on material in toto, as well as convenient for demonstrating cell structures which would be completely obscured by such a stain as Heidenhain's iron alum hematoxylin.

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